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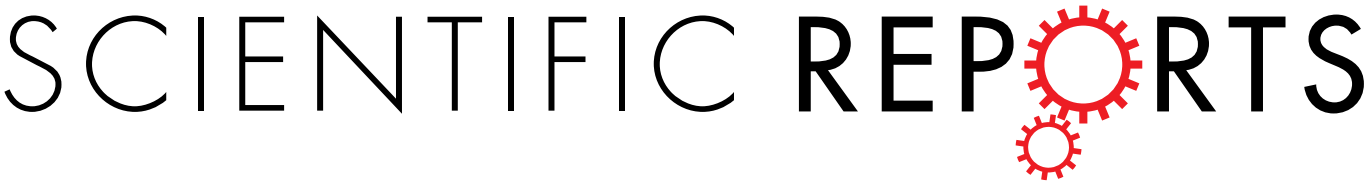

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Intermediate predator naïveté and sex-skewed vulnerability predict the impact of an invasive higher predator

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The spread of invasive species continues to reduce biodiversity across all regions and habitat types globally. However, invader impact prediction can be nebulous, and approaches often fail to integrate coupled direct and indirect invader effects. Here, we examine the ecological impacts of an invasive higher predator on lower trophic groups, further developing methodologies to more holistically quantify invader impact. We employ functional response (FR, resource use under different densities) and prey switching experiments to examine the trait- and density-mediated impacts of the invasive mosquitofish *Gambusia affinis* on an endemic intermediate predator *Lovenula raynerae* (Copepoda). *Lovenula raynerae* effectively consumed larval mosquitoes, but was naïve to mosquitofish cues, with attack rates and handling times of the intermediate predator unaffected by mosquitofish cue-treated water. Mosquitofish did not switch between male and female prey, consistently displaying a strong preference for female copepods. We thus demonstrate a lack of risk-reduction activity in the presence of invasive fish by *L. raynerae* and, in turn, high susceptibility of such intermediate trophic groups to invader impact. Further, we show that mosquitofish demonstrate sex-skewed predator selectivity towards intermediate predators of mosquito larvae, which may affect predator population demographics and, perversely, increase disease vector proliferations. We advocate the utility of FRs and prey switching combined to holistically quantify invasive species impact potential on native organisms at multiple trophic levels.

Invasive species incursions and proliferations are accelerating and present an enormous threat to environments and economies globally[1,2]. Freshwater ecosystems are particularly vulnerable to invasions due to high human-mediated propagule pressure and interconnectedness enabling rapid establishment and spread[3,4]. Indeed, anthropogenic modifications of freshwater systems, such as flow manipulation[5] and impoundment construction[6], can further heighten vulnerabilities to invaders[7,8]. Naïveté of native communities can exacerbate suppressive interactions with invasive species, especially in insular ecosystems (e.g. freshwaters) where there are no trophically analogous natives[9–12]. In particular, prey naïveté to unfamiliar cues or behaviours can profoundly increase impacts by invasive predators compared to native equivalents[13,14]. Reciprocally, naïveté can also influence biotic resistance between naïve native predators and invasive prey through processes such as prey preferences and switching with native prey[15–17]. However, invasion science has been slow to develop predictive methods to quantify invader impacts, and we currently lack quantitative approaches to forecast how prey naïveté and demography may affect invader impact strengths in recipient environments at multiple trophic levels.

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Invasive fishes have been especially damaging to freshwater ecosystems, driving extinctions of indigenous species[18]. Human-mediated introductions of fish into novel, previously fishless systems risk fundamentally altering species compositions and diversities through processes such as predation[19,20]. A key challenge therefore surrounds the quantification and prediction of invasive higher predator impacts on underlying trophic groups. These impacts can be profound[21], and may manifest in trophic cascades driven by both consumptive, density-mediated indirect interactions (DMIIs[22,23]), and non-consumptive, trait-mediated indirect interactions (TMIIs[23,24]). Critically, TMII effects may be as impactful as those resulting from direct consumption[24–26]. These effects can, in turn, be dependent on coevolutionary histories between trophic groups, or 'adaptive lag' of native assemblages[27], and aquatic systems present an ideal platform to examine indirect, TMII effects due to the prevalence and ease of manipulation of water-borne predator cues[28]. However, predicting impacts by invasive species on native prey can be complicated due to density- and context- dependencies, which may be non-additive in effect[29–31].

Functional responses (FRs) have been used extensively in the quantification of consumer-resource interactions, and FRs can be powerful tools to quantify density- and context-dependencies of invader impact[32–34]. Indeed, FRs can be applied to examine multiple predator effects between interacting con- and interspecific invasive species[21,30]. In the context of predation, the FR encapsulates prey consumption by predators in relation to prey density, with both FR form and magnitude powerful indicators for the derivation of consumer impact strengths[34]. Three common forms of FRs have been categorised: (1) Type I FRs are regarded as filter feeder-specific, with intake increasing linearly with prey availability[35]; (2) Type II FRs are characterised by a decelerating intake rate, which may be conducive to prey destabilisation as a result of high proportional consumption at low prey densities[34]; (3) Type III FRs are, in turn, characterised by low intake rate at low prey densities, and are sigmoidal in form[33], thus potentially imparting stability to prey populations by facilitating refugia for prey at low densities. The application of comparative FRs can not only be informative in terms of relative consumer impacts, but also directly enables the derivation of emergent context-dependencies that modulate consumer-resource interaction strengths[34,36]. These effects can be both abiotic (e.g. temperature/structural complexity[37]) and biotic (e.g. higher predators[21]). For instance, the detection of kairomones from a familiar higher predator can modify foraging intensity of intermediate predators towards basal prey[38]. This may manifest in modulations to the form and magnitude of FRs[28]. However, in cases where an intermediate predator is exposed to a novel threat, these responses may be nullified due to naïveté and, thus, predation vulnerability may not be alleviated.

Another classic concept within consumer-resource ecology surrounds prey switching, or frequency-dependence of predation[39]. Prey switching may be a powerful indicator when utilised alongside FRs to examine consumptive traits and impacts. However, prey switching has hitherto remained under-applied in invasion science, reducing our capacity to predict invader impacts (but see Cuthbert *et al.*[17]). Characteristically, when consumers exhibit a prey switching propensity, disproportionately more of the abundant prey type are consumed whilst disproportionately fewer rare prey are consumed[39]. This can foster stability in diverse prey populations, enabling coexistence patterns to emerge. Indeed, prey switching can be a key driver of the sigmoidal, stabilising Type III FR[40]. Furthermore, switching between intraspecific prey types can have demographic implications, particularly if prey consumption is sex-skewed. In turn, this can lead to emergent inequalities in sex ratios which may affect the population persistence of lower trophic groups[41,42]. As such, quantifying prey switching propensities between intraspecific prey forms can elucidate likely demographic and density-mediated outcomes for prey species following novel higher predator introductions.

The mosquitofish, *Gambusia affinis* (Baird and Girard), is one of the most widespread fish globally, having been introduced extensively in mosquito control efforts in recent decades[43]. Further, it is regarded as one of the world's worst invasive species[44], inducing negative impacts on native fish, amphibians and aquatic invertebrates[20,45,46]. The effectiveness of mosquitofish in biological control has been fundamentally questioned[47], and their application has been recorded to, perversely, increase mosquito proliferations due to interguild predation upon intermediate trophic groups such as notonectids[48]. In turn, this has resulted in calls to cease the use of such non-native fish in biological control efforts[49]. Furthermore, mosquitoes have been shown to comprise less than 1% of the diet of *G. affinis*, whilst zooplankton compose a majority[50], demonstrating generalist feeding strategies that reduce biological control efficacy of the mosquitofish. Yet, we currently lack holistic impact quantifications of such invasive species upon ecosystems outside of their native range.

In the present study, we thus use FR and prey switching experiments to quantify the impact of *G. affinis* on native trophic groups which are vulnerable to localised extinctions[19]. We examine the responsiveness of an intermediate predator, endemic to South Africa, the open-water calanoid copepod *Lovenula raynerae* Suárez-Morales, Wasserman and Dalu to water-borne mosquitofish cues, using mosquito larvae of the *Culex pipiens* complex as a basal prey. The *C. pipiens* mosquito complex is widespread globally, and colonises an extensive range of aquatic habitats, including temporary ponds. Calanoid copepods are also widespread and form an abundant and important component of freshwater ecosystems[51]. *Lovenula raynerae* is an ephemeral pond specialist species[52], and thus has evolved within fishless aquatic systems. Given a limited distribution, this copepod is highly vulnerable to environmental change. Indeed, mosquitofish have been documented to invade ephemeral systems[53,54], and *L. raynerae* have been detected in longstanding fishless systems where fish may persist if introduced (Wasserman pers. obs.). Thus, the potential for impact of mosquitofish on such vulnerable populations is high. Our approach examines responsiveness of *L. raynerae* consumption to visual and chemical mosquitofish cues and thus naïveté to predation by the novel invader. Additionally, we examine prey switching propensities of mosquitofish between female and male copepods, elucidating whether predation of *L. raynerae* by *G. affinis* will affect prey population viability through the establishment of sex-skewed ratios. Thus, we aim to illustrate the likely trait- and density-mediated impacts of the introduction of an invader on an intermediate predator and the cascade to its prey.

Chemical cue	Visual cue	First order term, *p*	*a*, *p*	*h*, *p*
No	No	−0.05, 0.001	0.66, 0.04	0.23, 0.002
Yes	No	−0.06, <0.001	1.24, 0.16	0.35, <0.001
No	Yes	−0.07, <0.001	0.80, 0.02	0.26, <0.001
Yes	Yes	−0.04, 0.005	0.54, 0.03	0.21, 0.005

Table 1. First order terms and significance levels resulting from logistic regression of proportion of prey eaten as a function of prey density, alongside FR parameter estimates across cue treatments with significance levels resulting from the Rogers' random predator equation in Experiment 1.

Results

Prey survival in controls exceeded 99% in both experiments, thus we assumed experimental deaths were due to predation, which we also observed directly. In Experiment 1, overall consumption by copepods was not significantly affected by the presence of *G. affinis* chemical cues ($\chi^2 = 0.09$, $df = 1$, $p = 0.76$), visual cues ($\chi^2 = 0.02$, $df = 1$, $p = 0.88$), or interaction between these cues ($\chi^2 = 0.10$, $df = 1$, $p = 0.76$). Overall prey consumption was significantly greater under increasing prey supplies ($\chi^2 = 30.61$, $df = 4$, $p < 0.001$). Further interactions among 'chemical cue', 'visual cue' and 'prey supply' were non-significant and thus were removed stepwise from the model.

As first order terms were significantly negative in each experimental treatment (Table 1), we deemed all FRs to be categorically Type II. Attack rates of *L. raynerae* did not differ significantly between cue-free and *G. affinis* cue treatments (chemical cue: $z = 0.63$, $p = 0.53$; visual cue: $z = 0.30$, $p = 0.76$; both cues: $z = 0.31$, $p = 0.76$), and there was no significant difference within cue treatments (all $p \geq 0.44$). Handling times of *L. raynerae* also did not vary significantly between cue-free and *G. affinis*-treated waters (chemical cue: $z = 0.99$, $p = 0.32$; visual cue: $z = 0.32$, $p = 0.75$; both cues: $z = 0.20$, $p = 0.84$), and there was, again, no significant difference within cue treatments (all $p \geq 0.24$). Confidence intervals overlapped amongst all FRs across the entire spectrum of prey supplies, further illustrating similarities in attack rate, handling time and, inversely, maximum feeding rate parameters between different cue treatments (Fig. 1).

In Experiment 2, mosquitofish displayed strong preference for female over male copepods at all prey proportions with the exception of extreme ratios (30:0, 0:30), wherein prey choice was necessarily restricted to one copepod sex (Table 2; Fig. 2). Thus, prey switching did not occur between male and female copepod prey, with preference for female copepods exhibited even when presented at relatively low proportions relative to males. Overall consumption was significantly greater for females than males ($F_{1,54} = 20.22$, $p < 0.001$), and was significantly affected by the proportion of prey available ($F_{6,48} = 10.89$, $p < 0.001$), with greater consumption for a specific prey type exhibited when it was available in higher proportions. There was no significant 'sex × proportion' interaction ($F_{6,42} = 1.01$, $p = 0.44$), and thus this interaction was removed from the model. Manly's α preference indices were significantly greater for females, suggesting an overall preference for this prey type ($\chi^2 = 31.17$, $df = 1$, $p < 0.001$; Table 2). Manly's α values were additionally significantly affected by the proportions of prey available ($\chi^2 = 58.82$, $df = 6$, $p < 0.001$), and there was a significant 'sex × proportion' interaction ($\chi^2 = 15.08$, $df = 6$, $p = 0.02$), with greater preference for females shown at intermediate prey ratios (Fig. 2).

Discussion

The identification of measures to understand and forecast invasive species impacts on recipient ecosystems is critical for biodiversity protection and developing proactive management approaches for invasions[34,55]. In our study system, we forecast trait- and density-mediated impacts of a widespread, invasive fish, the mosquitofish *G. affinis*, on an endemic intermediate predator, the calanoid copepod *L. raynerae*. We apply FR[32,33] and prey switching[42] approaches experimentally, showing firstly that the feeding magnitude of *L. raynerae* is not significantly affected by either chemical or visual cues of *G. affinis*. Secondly, our study highlights the much higher susceptibility of female over male *L. raynerae* copepods to *G. affinis* predation. Therefore, we show that the potential for invader impact is high, given that the invasive mosquitofish readily consumes and impacts populations of naïve intermediate predators of mosquito larvae, which may affect overall biotic resistance towards mosquito prey. In addition, invader impact may have implications for *L. raynerae* demographics as the copepod exhibits sex-skewed vulnerabilities to the invasive fish. These results are pertinent given that Wasserman *et al.*[41] showed, conversely, that natural predation on *L. raynerae* by common aquatic insects resulted in lower risk levels for females. Thus, augmented vertebrate predation through *G. affinis* introductions would likely have implications for *L. raynerae* population sex demographics in natural systems, having a further destabilising effect which may reduce population persistence of threatened endemic populations.

Predatory copepods, such as *L. raynerae*, often dominate small aquatic ecosystems which are of high importance for biodiversity in arid environments[56,57]. The small ecosystems which *L. raynerae* dominate function entirely differently to other aquatic systems, and are characterised by restricted higher trophic structuring[52]. Thus, populations within these habitats are especially vulnerable to augmented higher order predation through species introductions[19]. Given the orientation of this copepod to surface waters, vulnerabilities of the species to fish predation may be bolstered by indifferent foraging intensities in the presence of predator cues shown here, coupled with a pronounced association with the upper water column where mosquitofish forage[50]. Biotic contexts such as higher predator risk can have a substantial impact on predator-prey interaction strengths[21,47,58], but can often be dependent on coevolutionary context[10,27,59,60]. Indeed, invertebrates have been found to be generally responsive to higher predator cues arising from different diets[26]. Such responses frequently reduce predatory impacts exerted upon basal prey by intermediate predators[28]. Here, in contrast, we demonstrate naïveté of *L. raynerae* to

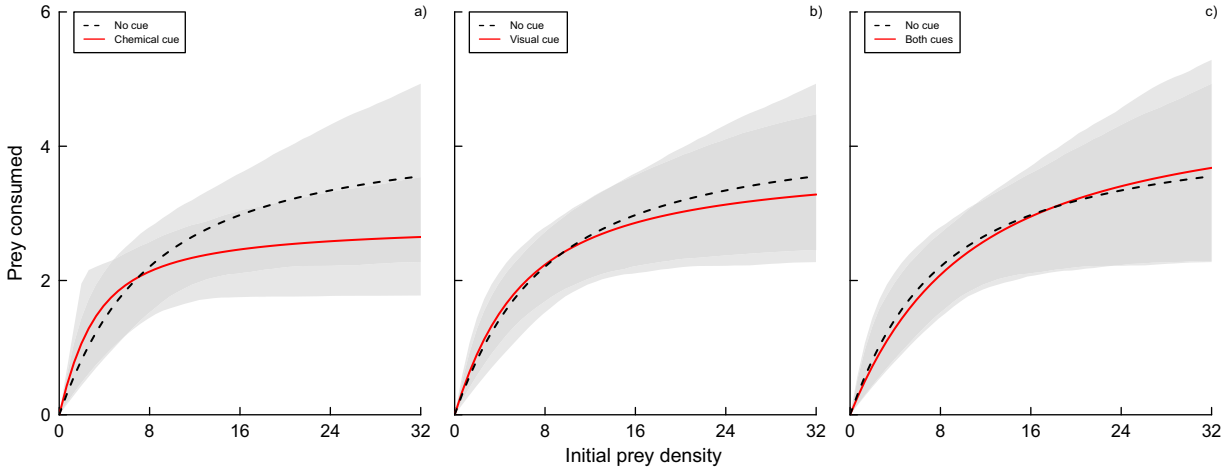

Figure 1. Functional responses of male *L. raynerae* towards larval culicid prey without cues of *G. affinis* compared to FRs in the presence of (**a**) chemical cues, (**b**) visual cues and (**c**) both cues. Shaded areas around FRs represent bootstrapped ($n = 2000$) confidence intervals.

unfamiliar predators, as indicated by the recurrence of Type II FRs and similarities in FR parameters (attack rates, handling times) between cue treatments. The exhibited Type II form here corroborates with results of Wasserman *et al.*[61] and Cuthbert *et al.*[62,63], where destabilising FRs of *L. raynerae* were also constrained with daphniids and culicids as a basal prey.

In addition to indirect interactions, selectivity by higher-order predators can have direct implications for the demographics of recipient ecosystems[41]. Higher male copepod vulnerability to predation has been recurrently hypothesised due to risks associated with mate-searching and copulation[64,65]. Indeed, Wasserman *et al.*[41] illustrated that predation of *L. raynerae* by native hexapods is selective towards males due to the processes of copulation. Here, however, we find the opposite in the presence of an invasive higher predator, with high, frequency-independent selectivity demonstrated towards females, which are larger and less motile than males (Cuthbert pers. obs.). The lack of prey switching exhibited here is indicative of an absence of prey refuge for female *L. raynerae* when available in lower proportions, which may have stark implications for demographics and the reproductive success in mature zooplankton populations following invasive fish introductions. The mechanisms of higher-order predatory pressure from fish operate entirely differently from invertebrates; where partial prey consumption is often exhibited by invertebrates, fish consume prey whole[41]. Therefore, the selective tendencies of higher-order fish predation towards females exhibited here may be compounded by the nullification of risk-evasion responses of females when copulating, with copulating pairs perceived, rather, as a single prey unit by fish. This is particularly relevant in light of the extended copulation period of *L. raynerae* and associated reduced instantaneous escape speed[41]. Thus, the introduction of invasive fish may fundamentally alter the demographics of prey populations in aquatic systems ecosystems previously dominated by invertebrates, potentially increasing extinction risk.

Conclusion

The spread of invasive species continues to circumvent biogeographical barriers and reduce biodiversity, and impacts on recipient communities can be intensified due to naïveté in recipient ecosystems[13,14]. Here, we illustrate, through the coupled use of experimental FR and prey switching approaches, that endemic intermediate predators in insular aquatic ecosystems are naïve to cues from the invasive mosquitofish *G. affinis*, and that selective predation by mosquitofish may affect the population structuring and persistence of native species. Furthermore, *G. affinis* will consume endemic intermediate predators of mosquito larvae that have themselves been suggested for use in mosquito biocontrol[62,63]. The frequency-independent preferences for female copepods demonstrated here by mosquitofish defies the selective preference for male copepods which has been typically posited[64,65]. Thus, the introduction of invasive mosquitofish for vector control could fundamentally shift the dynamics in recipient ecosystems, with effects on intermediate predators that potentially nullify or reverse attempts to control important vector mosquitoes through interguild predation[48]. We advocate that the use of FRs and prey switching offer robust and quantitative insights into the coupled direct and indirect impacts of invasive species on native populations. Prior examinations of such impacts could help to curtail damaging introductions, for instance through 'classical' biological control approaches which seek to release non-native agents into novel environments. Further research which incorporates multiple co-existing and interacting invaders alongside native biota would be of additional value in deciphering additive or non-additive trophic interactions within our framework.

Materials and Methods

Animal collection and maintenance. Ethical approval for experiments was granted by the animal ethics committee (AEC) within SAIAB (REF# 25/4/1/7/5_2017-14), in accordance with The South African National Standard for the Care and Use of Animals for Scientific Purpose (SANS 10386:2008). *Gambusia affinis* (34.7 ± 1.0 mm) were sourced from irrigation ponds within the Sundays River Valley, Eastern Cape, South Africa

Proportion supplied	Sex	Manly's α (±SE)
1.00	Female	1.00 (±0.00)
0.83	Female	0.73 (±0.16)
0.67	Female	0.75 (±0.14)
0.50	Female	0.92 (±0.05)
0.33	Female	0.68 (±0.12)
0.17	Female	0.63 (±0.21)
0.00	Female	0.00 (±0.00)
1.00	Male	1.00 (±0.00)
0.83	Male	0.37 (±0.21)
0.67	Male	0.32 (±0.12)
0.50	Male	0.08 (±0.05)
0.33	Male	0.25 (±0.14)
0.17	Male	0.27 (±0.16)
0.00	Male	0.00 (±0.00)

Table 2. Mean untransformed Manly's α preference index values for female or male *L. raynerae* displayed by *G. affinis* across varying proportions ($n = 4$ per treatment). Index values range from 0–1, with 0.5 indicating no preference and values closer to 1 indicating increasing preference.

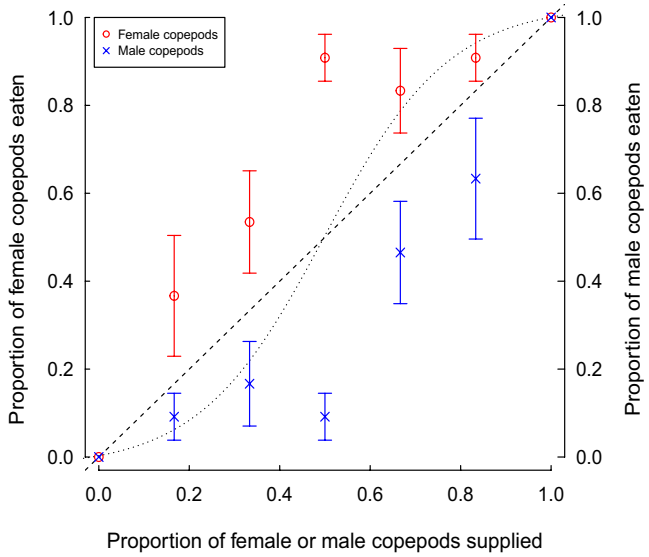

Figure 2. Proportion of female and male *L. raynerae* in diet of *G. affinis* as a function of the proportion supplied. The dashed line indicates the expected value if there was no preferential selection between the two prey types. The dotted sigmoid line represents a hypothetical switching pattern and means are ± standard error ($n = 4$ per group).

(33°26′23.38″S, 25°42′25.67″E) by seine netting in the austral summer 2017. Fish were transported in continuously aerated source water to a controlled environment room at Rhodes University, Grahamstown, maintained at 25.0 ± 1.0 °C and under a 14:10 light:dark regime. Fish were housed in continuously aerated 25 L aquaria containing dechlorinated tapwater and fed on a standard diet of *C. pipiens ad libitum* for at least 12 d prior to experimentation. *Lovenula raynerae* were collected from a pond in Grahamstown (33°16′47.8″S, 26°35′39.8″E), Eastern Cape, South Africa using a 200 μm mesh net and transported in source water to the same laboratory, and kept in 25 L aquaria containing continuously aerated water (matured tapwater and pond water, 50:50 ratio). Mosquito larvae were cultured using egg rafts collected from artificial containers within the Rhodes University campus, identified upon hatching and reared to the desired size class in the same laboratory using a diet of crushed rabbit pellets (Agricol, Port Elizabeth). Both predators were found to feed readily on larval mosquito prey.

Experimental design. We conducted two experiments to examine the impacts of the invasive fish *G. affinis* on the intermediate predator *L. raynerae*. Both experiments were undertaken in the environment room (25.0 ± 1.0 °C and under a 14:10 light:dark regime) using strained (20 μm), aerated water. In Experiment 1, individual adult male copepods (4.4 ± 0.1 mm) were selected for experimentation following collective starvation for 48 h and provided *C. pipiens* larvae (2.2 ± 0.1 mm) in transparent glass arenas of 5.6 cm diameter containing

80 mL water at five larval densities (2, 4, 8, 16, 32; $n = 4$ per density and treatment). The 80 mL inner experimental arenas were each placed within a larger opaque polypropylene outer arena of 16.5 cm diameter containing 800 mL water. We employed a fully factorial 2×2 experimental design with respect to predatory cues of *G. affinis*. Factor 1 comprised chemical cues (present/absent) and Factor 2 visual cues (present/absent). For chemical cues (Factor 1), a 2 L cue accumulation tank was established. In this tank, *G. affinis* were stocked at a density of 0.5 fish L^{-1} and left unfed for 48 h prior following the standard diet. The *G. affinis* treated water (cue water) was then used as the medium within the 80 mL experimental arenas. To implement visual cues (Factor 2), regular water was again used within the experimental arenas, but a single *G. affinis* was placed within the outer 800 mL arena and allowed to move freely, yet unable to consume the *L. raynerae* within the glass inner arena. Mosquito larvae and mosquitofish were added to the inner and outer arenas, respectively, two hours before the addition of the copepod predators and allowed to settle. Following their addition to the inner arena, copepods fed undisturbed for 6 h, after which they were removed and the remaining prey counted to derive those killed. Controls consisted of a replicate of all treatments in the absence of predators in order to constrain background mortality driven by processes outside of predation.

In Experiment 2, adult female and male copepods (female, 4.8 ± 0.1 mm; male, 4.4 ± 0.1 mm) were supplied at seven different ratios (30:0, 25:5, 20:10, 15:15, 10:20, 5:25, 0:30 individuals; $n = 4$ per ratio) to *G. affinis*, which had been starved for 24 h. These ratios reflect the varying proportions of *L. raynerae* in aquatic ecosystems (see Wasserman *et al.*[41]). Experiments were undertaken in arenas of 16.5 cm diameter containing 2 L water from a continuously aerated source. Once introduced, fish fed undisturbed for 3 h, after which they were removed and remaining living copepods counted and sexed. Controls consisted of a replicate at all treatments in the absence of predators.

Statistical analyses. All statistical analyses were undertaken in R v3.4.2[66]. In Experiment 1, generalised linear models (GLMs) assuming a Poisson error distribution were used to analyse overall prey consumption with respect to 'chemical cue', 'visual cue' and 'prey supply', and their interactions. Non-significant terms and interactions were removed stepwise from the model to facilitate parsimony, with χ^2 used for model simplification *via* analysis of deviance[67]. Functional response (FR) analyses were undertaken using the 'frair' package in R[68]. Logistic regression of the proportion of prey consumed as a function of prey density was used to infer FR types. A Type II FR is characterised by a significantly negative first order term, whilst a Type III FR is characterised by a significantly positive first order term followed by a significantly negative second order term[32,33,69,70]. As prey were not replaced as they were consumed, we applied Rogers' random predator equation for depleting prey densities[69,70]:

$$N_e = N_0(1 - \exp(a\,(N_e h - T))) \tag{1}$$

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant, h is the handling time and T is the total experimental period. The Lambert W function was used to enable model fitting[71]. We used the 'difference method'[70] to compare attack rate and handling time parameters generated from FRs across treatments. To account for multiplicity, we compared coefficients against Bonferroni-adjusted p-values. Functional responses were non-parametrically bootstrapped ($n = 2000$) to generate confidence intervals, allowing the FRs to be considered in population terms[68].

In Experiment 2, as residuals were overdispersed, GLMs assuming a quasi-Poisson error distribution were used to compare overall prey consumption with respect to 'sex' and 'proportion', with F-tests used for model simplification. Again, non-significant terms and interactions were removed stepwise[67]. Manly's α[72,73] assuming no prey replacement was used to determine prey preferences between prey across the varying provision ratios:

$$\alpha_i = (\ln((n_{i0} - r_i)/n_{i0}))/\sum_{j=1}^{m}(\ln((n_{j0} - r_j)/n_{j0})) \tag{2}$$

where a_i is Manly's selectivity index for prey type i, n_{i0} is the number of prey type i available at the start of the experiment, r_i is the number of prey type i consumed, m the number of prey types, n_{j0} the number of prey type j available at the start of the experiment and r_j is the number of prey type j consumed. The value of α_i ranges from 0 to 1, with 0 indicating complete avoidance and 1 indicating complete positive selection. In a two-prey system, values of 0.5 are indicative of null preference. Manly's α indices were transformed to reduce extremes[74] (0 s, 1 s) prior to analysis:

$$a_t = (\alpha_i(n - 1) + 0.5)/n \tag{3}$$

where α_t is the transformed output and n is the sample size. Beta regression using the 'betareg' package[75] in R was used to compare Manly's α values between 'sex' and 'proportion', and their interactions. Akaike's Information Criterion was used to confirm that models minimised information loss (lower values indicate a better fit).

Data Availability

Raw functional response and prey switching data are available in the electronic supplementary material.

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Author Contributions

Conceived and designed the experiments: R.C., T.D., R.W., J.D., O.W. Performed the experiments: R.C., T.D., R.W. Analysed the data: R.C. Contributed materials and analysis tools: R.C., T.D., L.M., A.C., O.W. Wrote the first draft of paper: R.C. Prepared revised versions: R.C., T.D., R.W., J.D., L.M., A.C., O.W.

Additional Information

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